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Note

Rapid assay of tinidazole in plasma by high-performance liquid chromatography

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Tinidazole (Fasigyn®, Pfizer), ethyl 2-(2-methyl-5-nitro-1-imidazolyl) ethyl sulphone, is used in the treatment of trichomoniasis in man. For the measurement of nitroimidazole-related compounds in human plasma, polarographic methods based on that described by Kane [1] have been used [2]. De Silva et al. [3] described methods for the determination of N-substituted nitroimidazole in blood and urine which involved a preliminary separation of unchanged drug by thin-layer chromatography (TLC) before quantitation by an absorptiometric, polarographic or gas chromatographic procedure. This TLC procedure was improved by Welling and Monro [4]: tinidazole was quantitated at the TLC stage by measuring the quenching of plate fluorescence. This method has been used for pharmacokinetic studies both in laboratory animals [5] and in man [4, 6]. However, the TLC procedure remains lengthy and is difficult to use for serial analyses of large number of samples.

In this paper a new high-performance liquid chromatographic (HPLC) assay is described which is quicker than the TLC method and no less specific.

EXPERIMENTAL

Instrumentation

Chromatography was performed on a Model 841 high-performance liquid chromatograph (DuPont) equipped with the Model 837 variable wavelength spectrophotometric detector set at 315 nm. Samples were injected through a Valco sample valve set for 50 μl volumes. The detector was connected to a Model 3380A recorder—integrator (Hewlett-Packard).

The chromatographic column was a stainless steel tube (1 m × 2.2 mm I.D.) filled with ETH Permaphase (25—37 μm; DuPont, Wilmington, Del., U.S.A.).

Reagents

All reagents were of analytical grade and used without further purification: chloroform RP (Prolabo, Paris, France) containing 0.5% ethanol as stabilizer, hexane (Merck, Darmstadt, G.F.R.), ethanol RP (Prolabo).

Assay prccedure

0.3 ml of plasma in a 12-ml centrifuge tube is diluted to 1 ml with water. After addition of 3 ml chloroform the tube is shaken for 20 sec on a Vortex mixer. The tube is then centrifuged for 5 min at 5000 *g* and the lower layer is transferred to a second 12-ml tube. The extraction is repeated once. The combined organic extracts are evaporated to dryness under a stream of nitrogen at 40°. The dry residue is dissolved in 0.75 ml of a hexane—chloroform—ethanol (90:15:0.5) mixture and 50 µl are injected into the chromatographic column.

The above mixture is used as mobile phase and pumped at a constant flow-rate of about 1 ml/min under a pressure of about 450 p.s.i. at room temperature. The retention time of tinidazole is about 3 min.

Two injections of each extract are performed.

Calibration

Each day, calibration samples are prepared by adding up to 700 µl of a suitable aqueous solution of tinidazole to 0.3 ml plasma. Three or four calibration samples containing 2—10 µg of tinidazole are prepared.

RESULTS AND DISCUSSION

Typical chromatograms are shown in Fig. 1. The first peak corresponds to normal plasma constituents and is relatively constant irrespective of the time elapsed between tinidazole administration and blood sampling. No other peak was observed after that of tinidazole.

Comparing tinidazole peak areas from organic solutions to those from extracts of spiked plasma, the recovery of the extraction process was close to 99% whatever the concentration used. The coefficient of variation determined

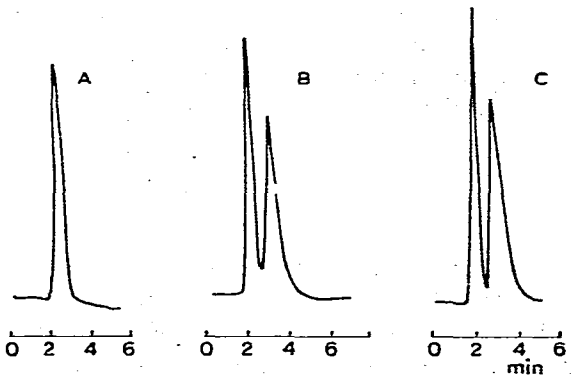

Fig. 1. A, Chromatogram of a blank plasma extract. B, Chromatogram of an extract of plasma spiked with tinidazole (5 µg/ml). C, Chromatogram of an extract of plasma from a volunteer after tinidazole administration. For HPLC conditions, see text.

from peak areas of four different assays on the same samples was about 4%. The minimum detectable concentration using 0.3-ml plasma aliquots was about 0.2 μg/ml, whereas the minimum measurable concentration was about 1 μg/ml. This sensitivity can be improved using higher plasma volumes up to 1 ml without peak overlap. The present conditions are satisfactory, however, since the range of tinidazole plasma levels encountered in clinical use is far above 1 μg/ml. Both recovery of the extraction and linearity of reference curves are satisfactory up to concentrations as high as 100 μg/ml.

As shown in Fig. 2, a good correlation between the present HPLC assay and the TLC procedure of Welling and Monro [4] was obtained. This suggests that the two techniques exhibit similar specificity, the HPLC method, however, being faster and less tedious: about 30 samples/day can be processed by one technician.

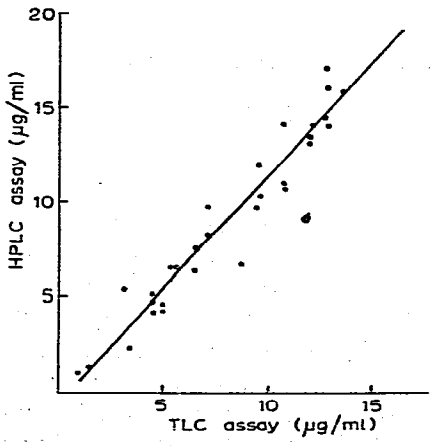

Fig. 2. Correlations between HPLC and TLC tinidazole assays in human plasma obtained at various time intervals after a single pessary (100 mg) administration.

This method has also been used successfully for assay of tinidazole in dog plasma; in this case, the first peak is higher than in human plasma but does not overlap with the tinidazole peak.

A similar chromatographic pattern was observed with metronidazole, suggesting that the present procedure could also be used for assay of metronidazole in plasma.

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